Digital PCR in the detection of HER2 copy number variation

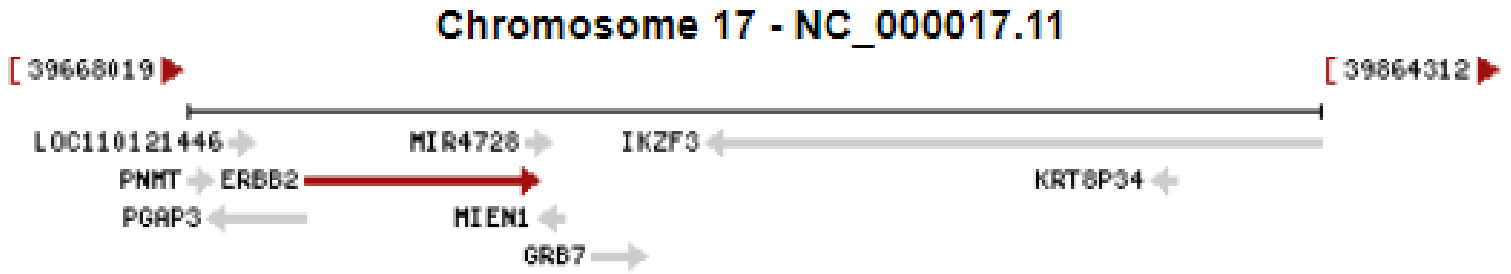
Contents

HER-2Gene

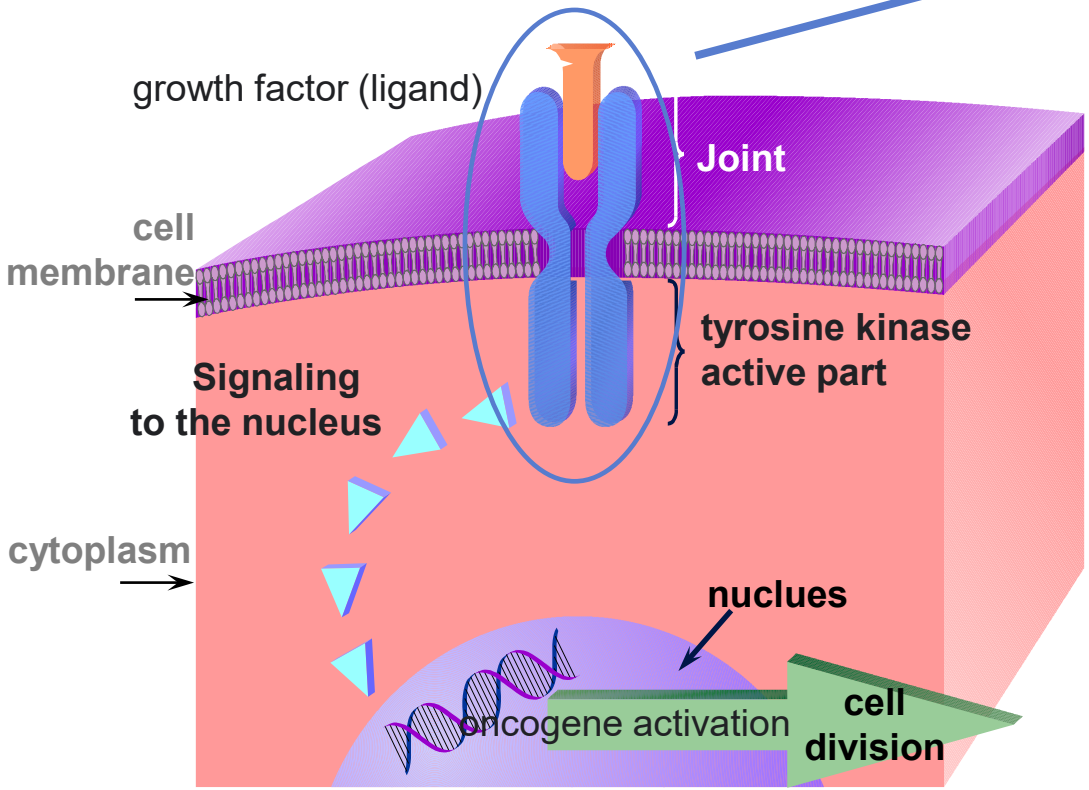
- **Human Epidermal growth factor Receptor** is a family of highly homologous proteins with tyrosine kinase activity. Members of this family include HER-1 (EGFR), HER-2, HER-3 and HER-4
- The EGER (also known as HER/ERBB) family activates tyrosine kinase activity and initiates cell growth signal transmission by being stimulated by specific types of growth factors
- Enhancement of growth signals can lead to malignant transformation of cells. The enhancement of growth signals is mainly due to the overexpression of receptor proteins, mutations of signaling proteins, and the continuous autocrine growth factors supplemented by the tumor itself.
- HER-2 often dimerizes with growth factor receptor proteins such as EGFR, and heterodimerization is one of the characteristics of the EGFR family

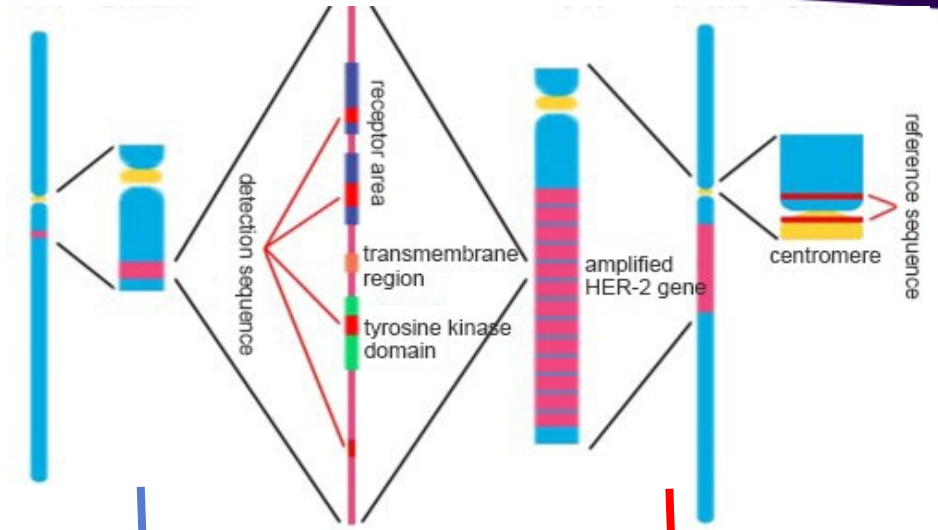
Breast cancer is the most common cancer occurring in women worldwide, with more than 1 million new cases each year.

HER2 encodes a membrane receptor tyrosine kinase, and its expression or amplification status is an important indicator for the prognosis and treatment of breast cancer patients. **20% to 30% of breast cancer patients** are caused by the amplification of the HER-2 gene on the chromosome of cancer cells. HER-2 protein is overexpressed on the cell membrane and is an independent prognostic factor for breast cancer.

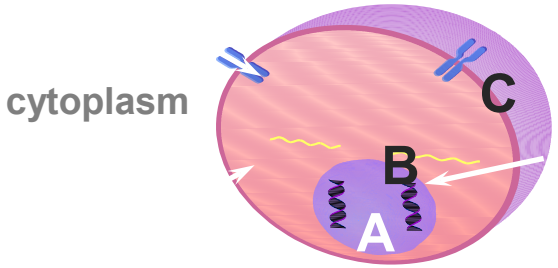


Signaling pathway of HER-2 receptor transmembrane dimer



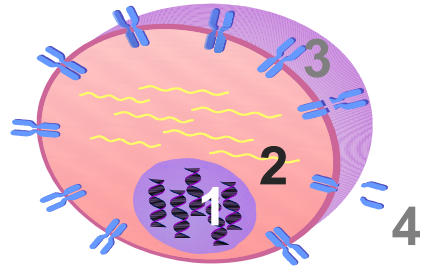


Normal



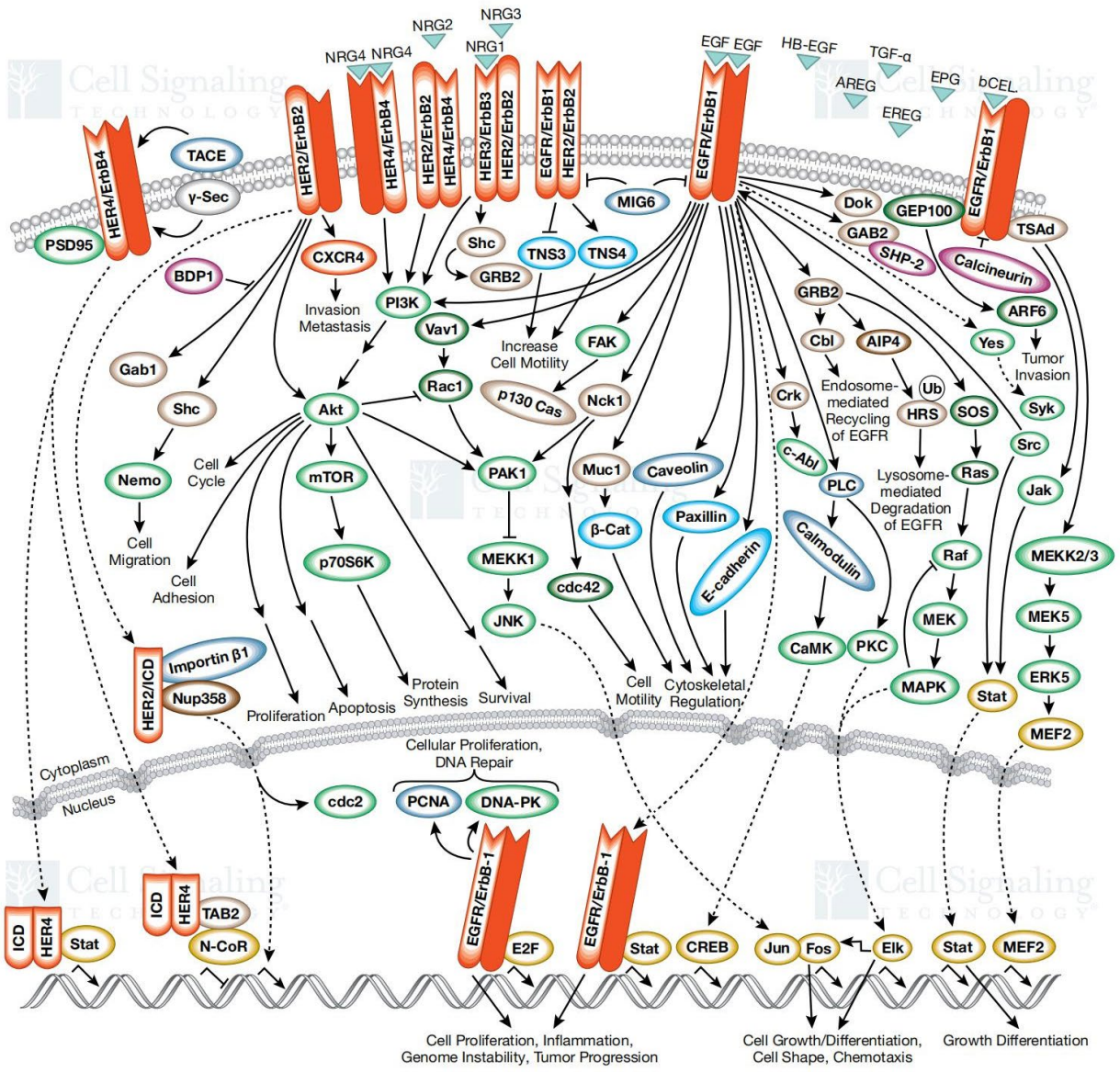
A = HER2 DNA
B = HER2 mRNA
C = HER2 receptor protein

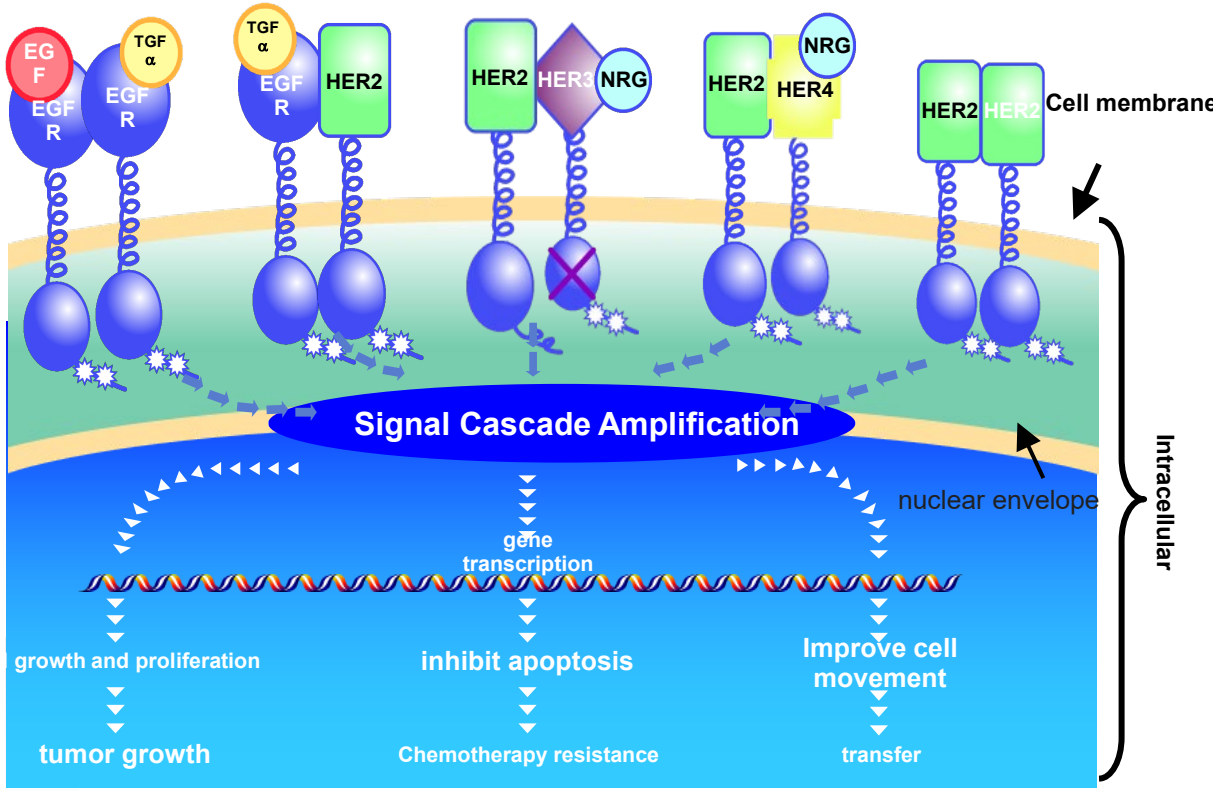
Amplification/overexpression



1 = gene copy number
2 = mRNA transcription
3 = cell surface receptor protein expression
4 = extracellular receptor domain release

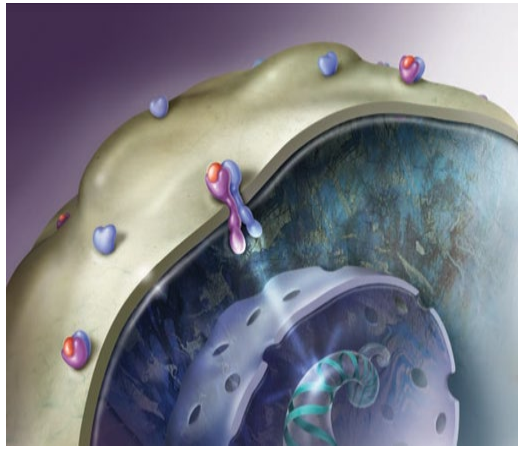
HER family - HER dimers trigger signaling



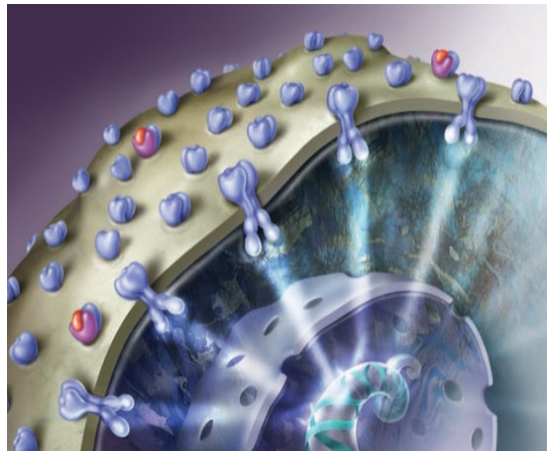


Yarden Y and Sliwkowsk MX. *Nat Rev Mol Cell Biol* 2001;2:127–137.

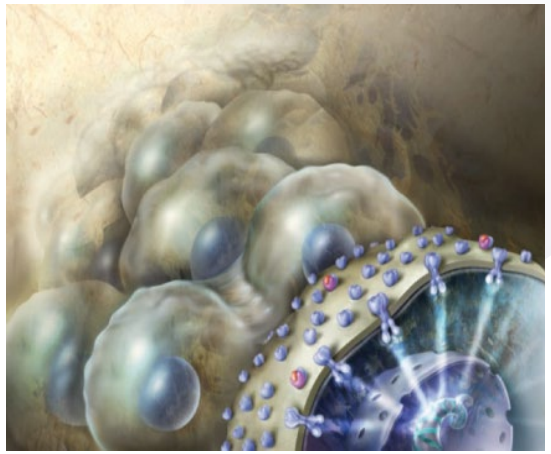
Results of HER2 overexpression



Normal HER2 expression

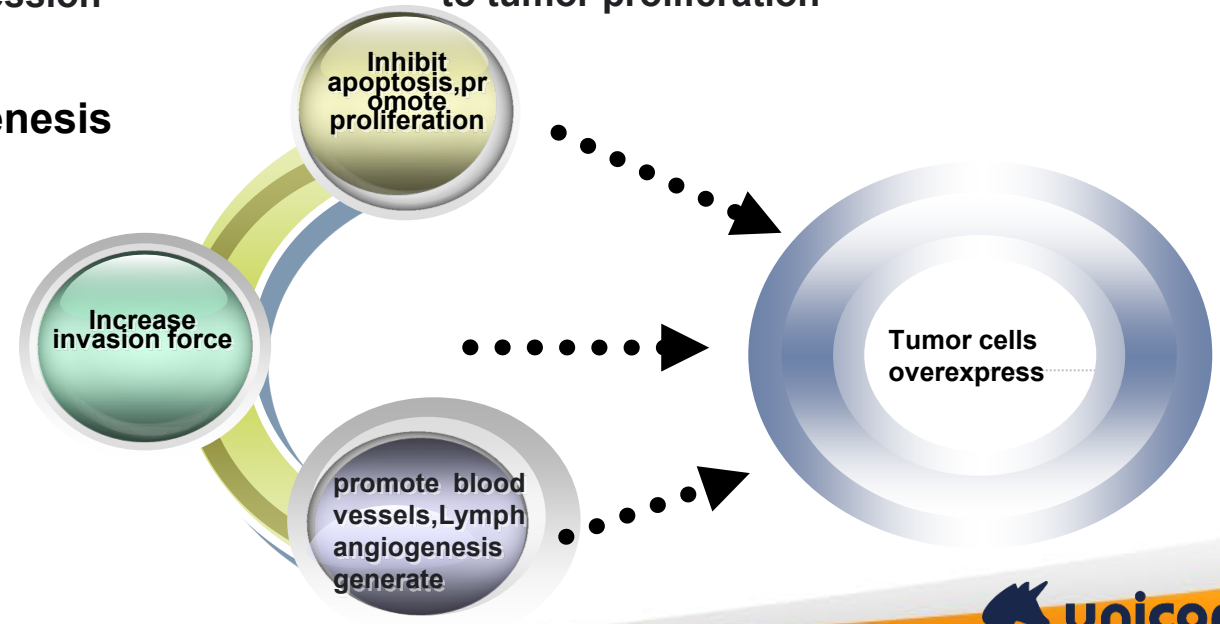


HER2 gene amplification leads to HER2 overexpression



HER2 overexpression leads to tumor proliferation

- Promote cell growth and proliferation --- tumorigenesis
- inhibit cell apoptosis
- induce angiogenesis
- Improve cell motility, enhance tumor invasion and metastasis



(1) Moasser MM.Oncogene,2007.26(46):6577
(2) Freudenbery JA, Wang Q, Katsumata, et al. Exp Mol Pathol.2009.87(1):1

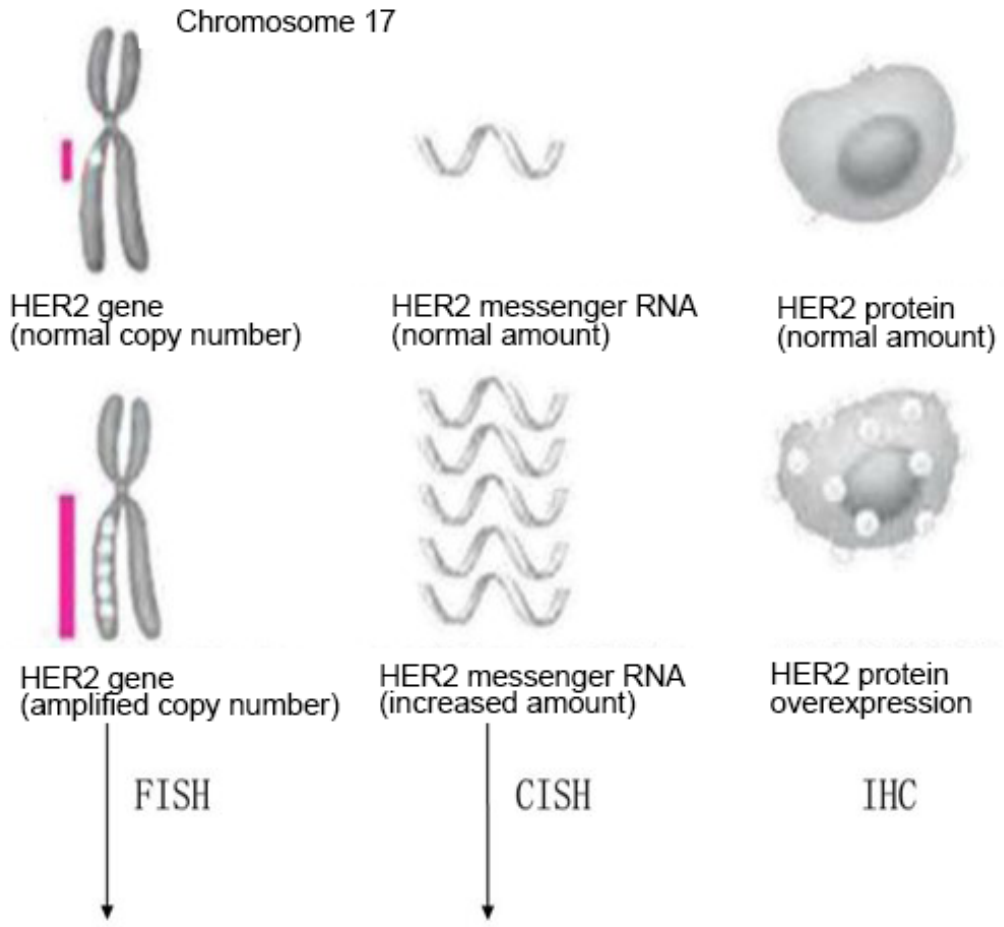
02 Breast cancer HER2 testing guidelines

- "Guidelines for HER2 Detection in Breast Cancer (2019 Edition)" still recommends a detection strategy combining IHC with FISH and/or CISH

- **IHC** (immunohistochemistry) directly targets HER-2 on the cell surface, which is fast, cheap and the closest to the truth. Usually lack of reference standards and require high samples

- **FISH** (fluorescence in situ hybridization) is the "gold standard" for large fragments of DNA, but detection is time-consuming, equipment-intensive and expensive. There may still be spurious results

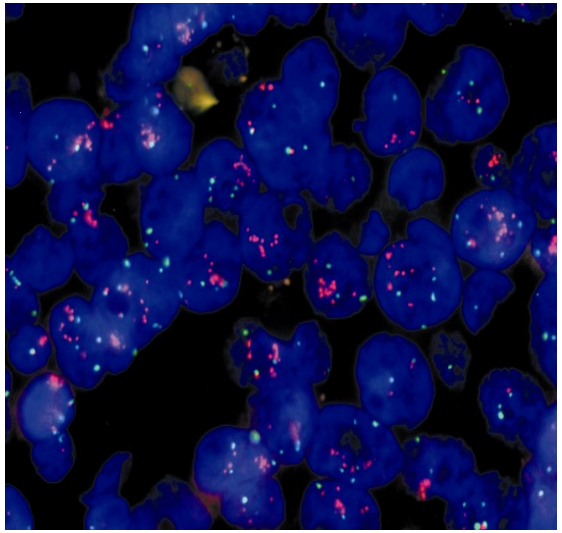
- **CISH** (chrome in situ hybridization) targets mRNA, which can better reflect the degree of protein expression than FISH, but it is unstable. There is a lack of intuitive control due to the single signal.



HER-2 assay comparison

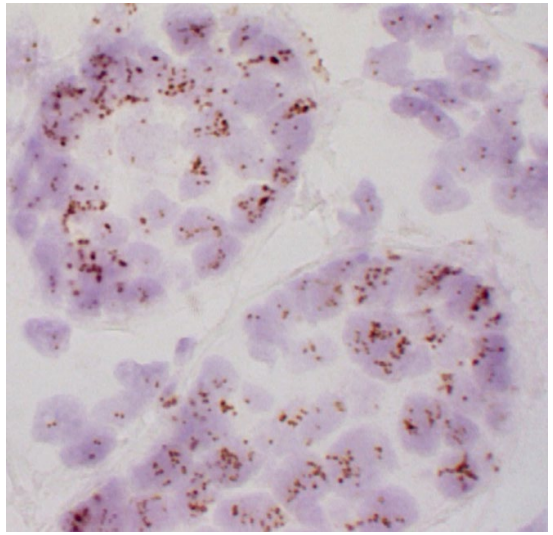
detection of HER2 gene amplification level

Chromogenic in situ hybridization (FISH)



•Accurate, repeatable and well correlated with curative effect
•Fluorescence microscope and other equipment are required
• Experienced operator needed
•The detection fee is higher 3000 RMB/case
•Few testing units in China

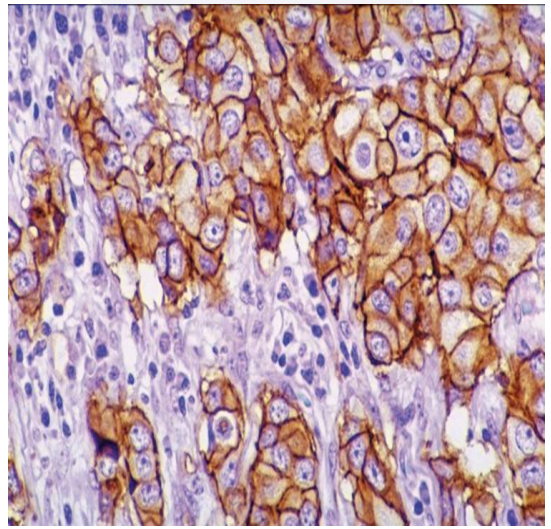
Fluorescence in situ hybridization (CISH)



•Operation and interpretation methods are similar to IHC
•Simultaneous histological evaluation
•High correlation with FISH test results
•Many centers that can carry out
•The detection cost is about 1500 RMB/case

Immunohistochemistry (IHC)

detection of HER2 receptor protein overexpression



•mature technology
•Fast simultaneous access to many case results
•Easier reading
•Cost 80~120RMB/case

Breast cancer HER2 detection guidelines, Chinese Journal of Pathology，2009;38(12):1-4.

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Breast Cancer HER2 Testing Guidelines (2019 edition)

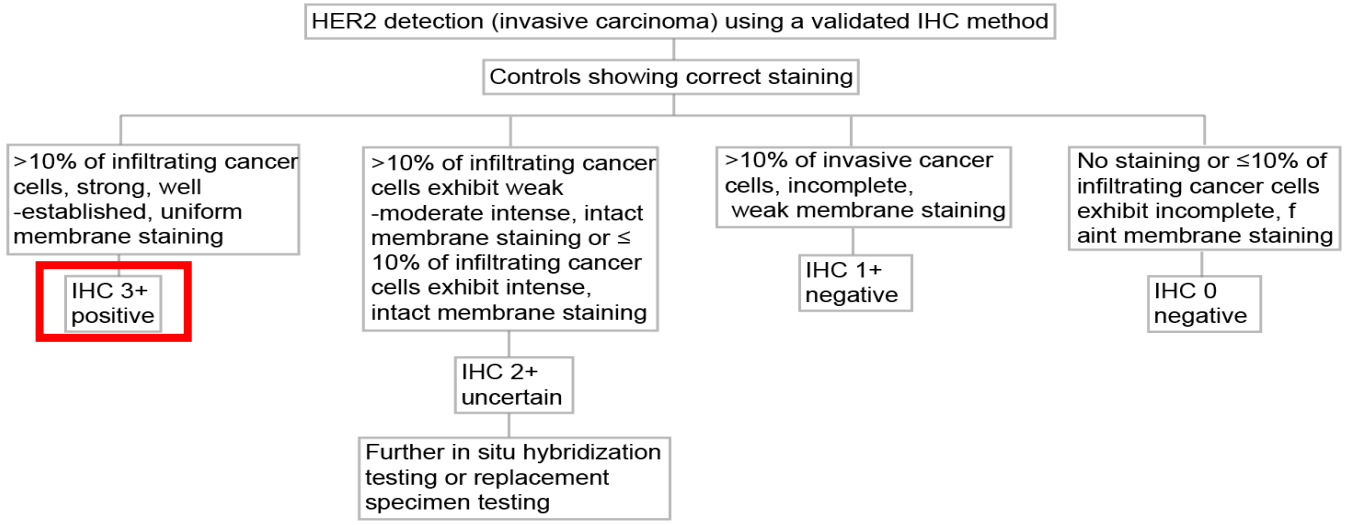


Figure1: HER2 immunohistochemical detection criteria

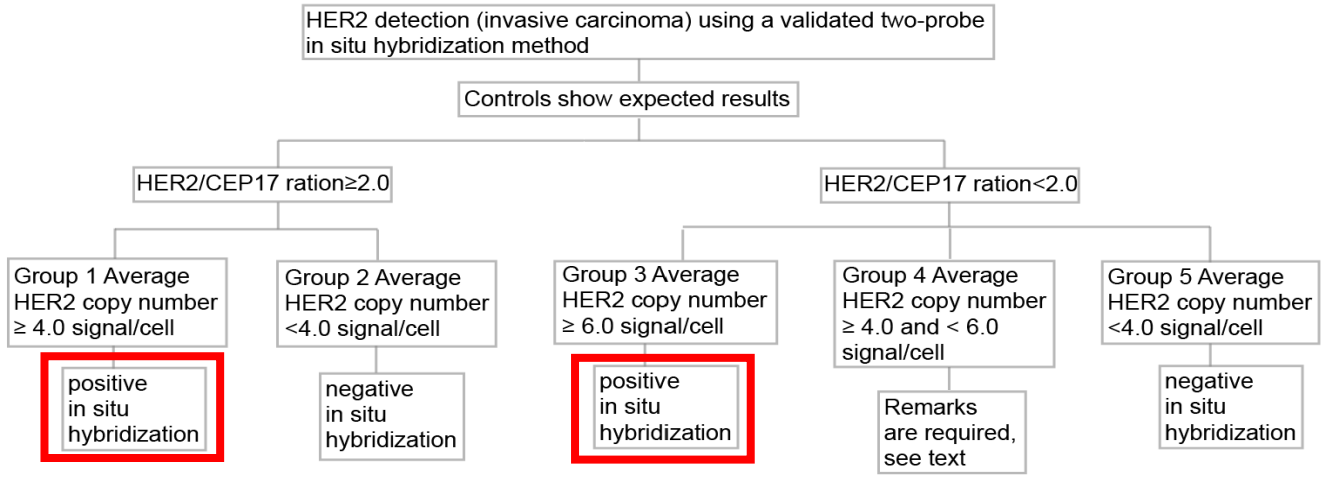
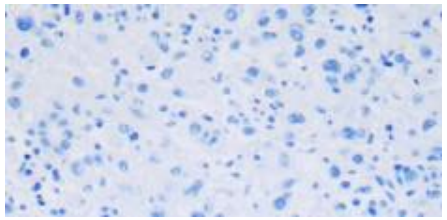


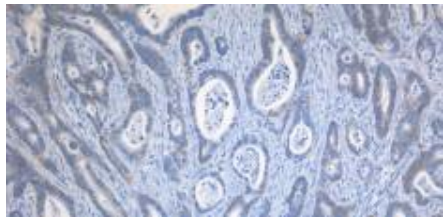
Figure2: HER2 dual-probe in situ hybridization detection criteria

Chin J Pathol, March 2019, Vol. 48, No. 3

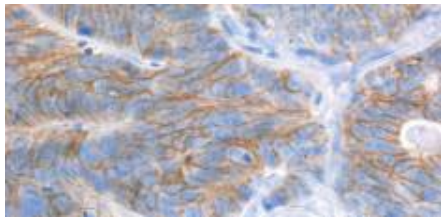
IHC Scoring Criteria



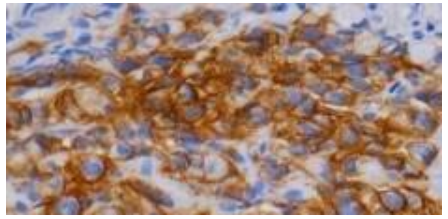
IHC negative (0)
No staining or <10% membrane staining



IHC negative (1+)
Faint/barely visible staining of >10% membranes; or only partial membrane staining



IHC suspicious (2+)
Mild to moderate membrane intact or basolateral membrane staining in >10% of cells



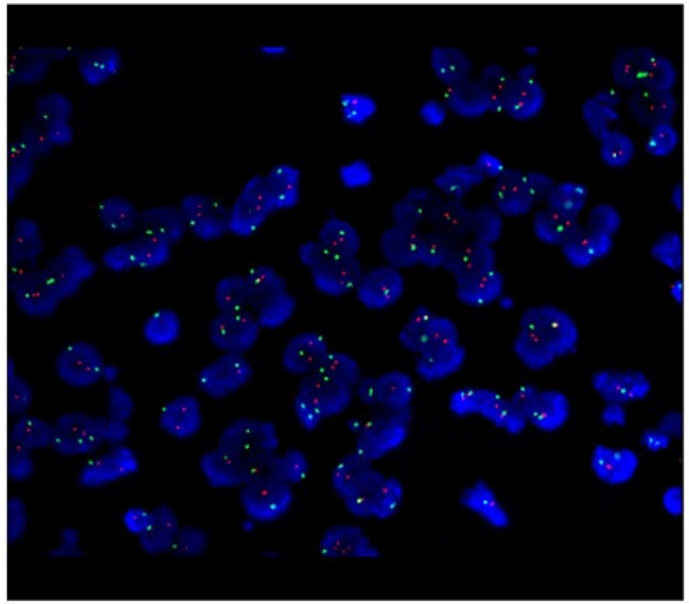
IHC positive (3+)
Strong staining of intact cell membrane or basolateral cell membrane with one of the following two conditions:

- In the resection specimen, the proportion of stained cells is >10%
- Colonies of strongly stained cells that aggregate regardless of the percentage of stained cells (eg, <10%) in the biopsy specimen

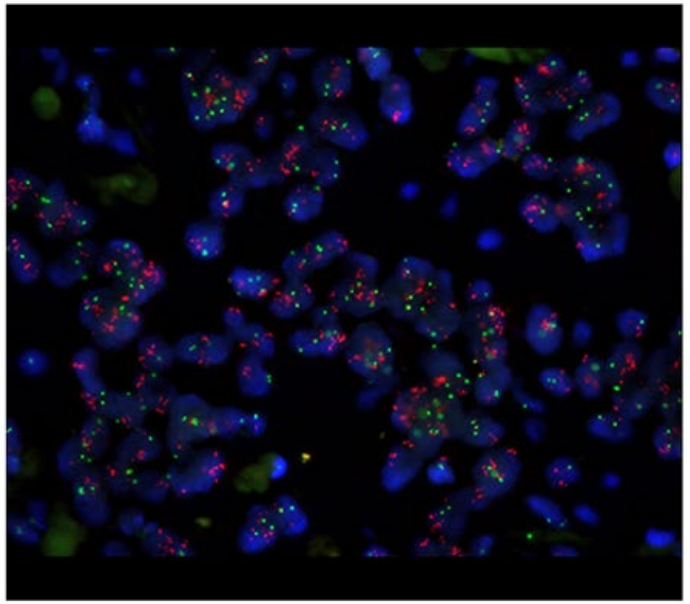
Staining depth \ Stained area	> 10%	⩽10%
strong staining	IHC 3+	IHC 2+
weak-moderately strong staining	IHC 2+	IHC 0
weak staining	IHC1+	IHC 0

Image from TARGOS

HER2 FISH detection interpretation criteria:



No amplification of HER2 gene

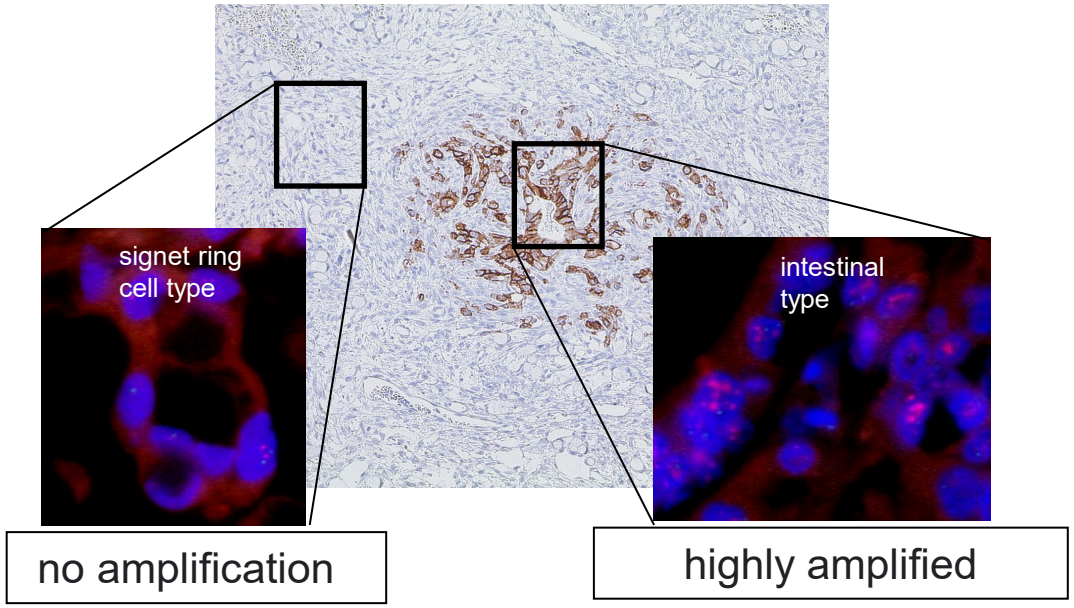


HER2 gene amplification

20 tumor cells, orange represents HER2 signal, green represents CEP17 signal

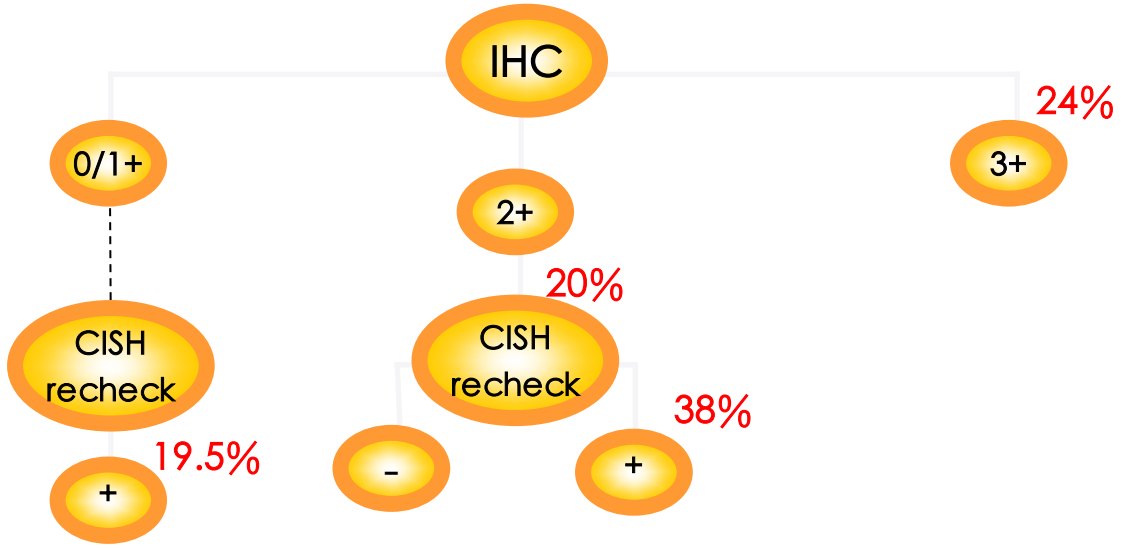
- HER2/CEP17≥2.0, indicating positive HER2 amplification.
- HER2/CEP17<2.0, and the average HER2 gene copy number ≥6.0 also indicates positive HER2 amplification.
- HER2/CEP17<2.0, and the average HER2 gene copy number<4.0, suggesting negative HER2 gene amplification.
- For cases with HER2/CEP17 ratio <2.0 and average HER2 gene copy number ≥4.0 and <6.0, HER2 amplification is uncertain.

Tumor heterogeneity



The inconsistency rate of HER2 expression in primary tumors and metastases of breast cancer is as high as 18.7%, and it is more obvious when the tumor is large or there are multiple tumors in the body. In breast cancer patients with distant metastasis, the HER2 amplification status Discordance rate between primary and metastases is as high as 30.0%

Breast Cancer Res Treat, 2009, 113(2): 301-306.
Chinese Journal of Oncology, 2018, 40(7): 506-511.



Summary of the research results of 270 prospective breast cancer cases in 5 centers, Fudan University Cancer Hospital, Zhongshan Medical University Cancer Hospital, Zhejiang University Second Affiliated Hospital, Soochow University First Affiliated Hospital, and 307 Hospital

·指南与共识·

外周血*HER2*基因扩增检测（数字PCR法）在抗HER2治疗中的应用共识

卢仁泉，柳光宇，杨文涛，郭　林，关　明，邵志敏，徐大志，倪　明，唐　峰，王朝夫，娄加陶，孙奋勇，邢金良，潘跃银

Passage: Consensus on the application of peripheral blood HER2 gene amplification detection (digital PCR method) in anti-HER2 therapy

［关键词］人表皮生长因子受体2；基因扩增；数字聚合酶链反应；共识
DOI: 10.19401/j.cnki.1007-3639.2022.01.012
中图分类号：R737.9　　文献标志码：A　　文章编号：1007-3639(2022)01-0090-07

Expert advice:

① The HER2 gene amplification detection kit (dPCR method) and the gold standard for tissue sample detection (IHC combined with FISH) have the same effect on the detection of tissue samples, and are also suitable for the detection of HER2 status in tissue samples of breast cancer patients.

② For newly diagnosed advanced (stage III, IV) breast cancer patients, when the tissue test is negative or the tissue sample is not available, the HER2 gene amplification detection kit (dPCR method) can be used for detection in peripheral blood. Patients with postoperative recurrent or metastatic breast cancer can also use the HER2 gene amplification detection kit (dPCR method) to detect in peripheral blood. The test results provide a reference for doctors to find possible treatment options for patients and avoid missing treatment opportunities.

Digital PCR, an effective supplement to HER2 gene amplification detection method

In addition to the challenges of FISH testing itself, which can cause false negative results in tissue biopsies, the spatiotemporal heterogeneity of tumors determines that HER2 gene amplification tissue biopsies may not fully reflect the status of HER2 genes in tumors, and because of the limitations of puncture, it also leads to Multiple tissue biopsies could not be performed as the patient's treatment progressed.

As a new clinical detection method, dPCR has brought new opportunities to detect HER2 amplification at the DNA level.

- Absolute quantitative performance and **excellent detection precision** of HER2 gene amplification detection (dPCR method)
- HER2 gene amplification detection (dPCR method) can not only solve the false negative results caused by irregular sample pretreatment, but more importantly, it is an effective supplement to the detection of tissue samples. To avoid false negative results due to spatio-temporal heterogeneity by **1 or more tests** on peripheral blood samples
- HER2 gene amplification detection (dPCR method) **dynamically monitors** the changes of HER2 amplification through the detection results of peripheral blood samples, which can be earlier than the evaluation criteria of solid tumor efficacy.

"Chinese Journal of Cancer", Volume 32, Issue 1, 2022 95（Response Evaluation Criteria in Solid Tumor，RECIST）

Tab. 1 Consistency analysis between the results of peripheral blood samples detected by dPCR and the results of homologous tissue IHC combined with FISH

Detection result of dPCR	Detection results of gold standard for tissue sample detection (ICH combined with FISH)		Total
	Positive	Negative	
Positive	42	20	62
Negative	54	106	160
Total	96	126	222

Positive coincidence rate 43.75%
Negative coincidence rate 84.38%
Total compliance rate 66.96%

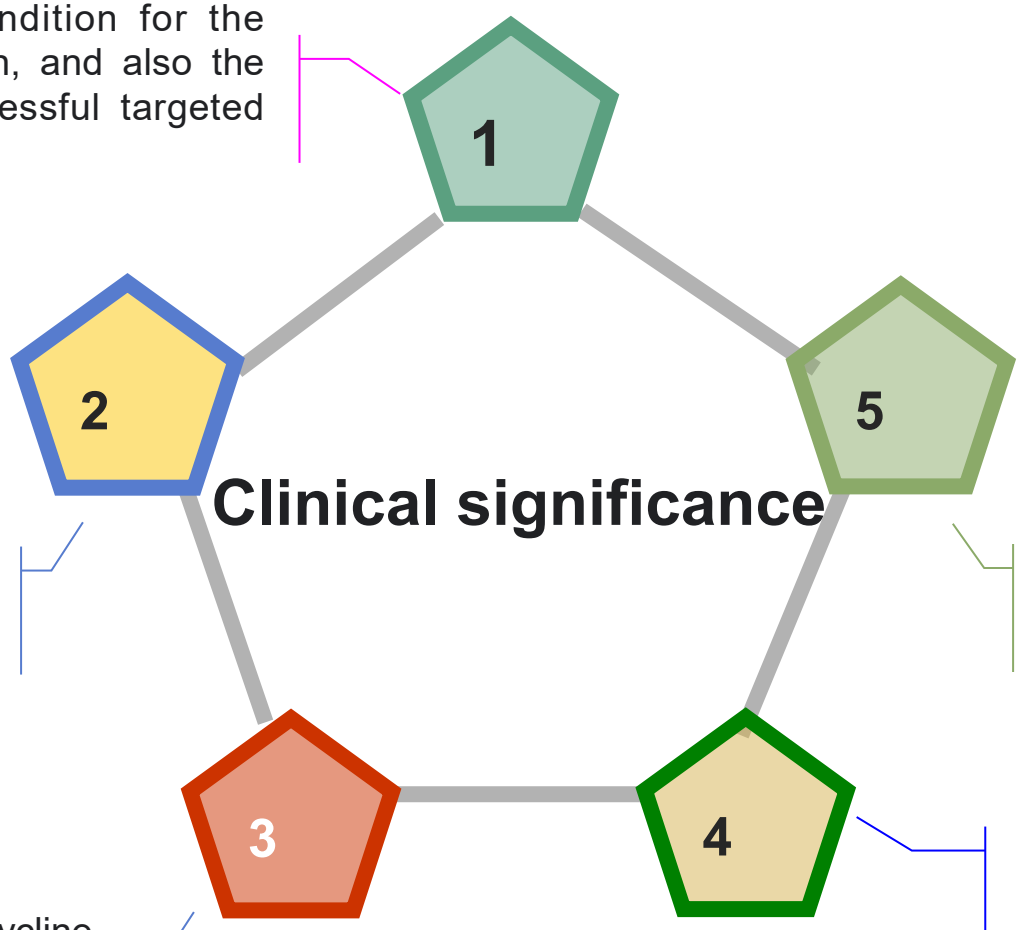
Tab. 2 Consistency analysis of peripheral blood samples detected by dPCR and homologous tissue IHC combined with FISH in patients with different disease stages

Item	Newly diagnosed stage Ⅲ patients (n=70)	Newly diagnosed stage Ⅳ patients (n=93)	Patients with recurrence and metastasis (n=59)
Sensitivity	37.93% (11/29)	41.67% (15/36)	51.61% (16/31)
Specificity	92.68% (38/41)	85.96% (49/57)	67.86% (19/28)
Total consistent rate	70.00% (49/70)	68.82% (64/93)	59.32% (35/59)
Kappa	0.331 2	0.296 0	0.192 7

03 Clinical Significance of HER2 Detection in Breast Cancer

Clinical significance of HER2 status in breast cancer

An absolutely necessary condition for the clinical application of Heceptin, and also the prerequisite and key to successful targeted therapy



It suggests that the degree of malignancy is high, rapid progression, and short remission period of chemotherapy

A reference index for prognostic judgment together with positive lymph nodes, high-grade cancer, negative receptor status, and high proliferative activity

Screening out candidates for anthracycline-based chemotherapy regimens

Strong basis for formulating endocrine treatment plan

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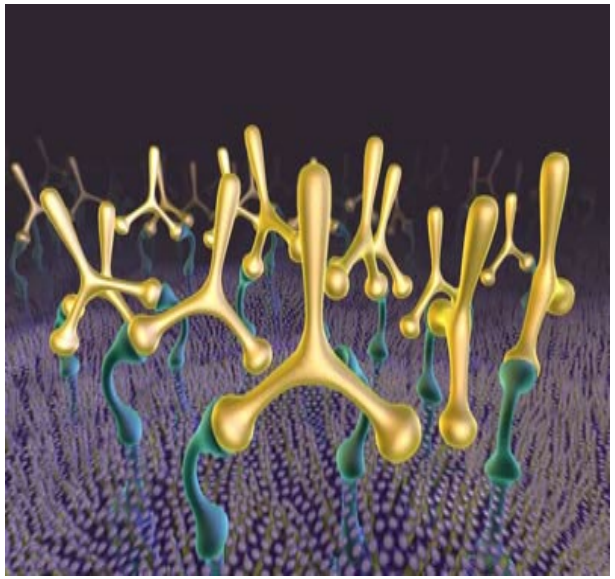
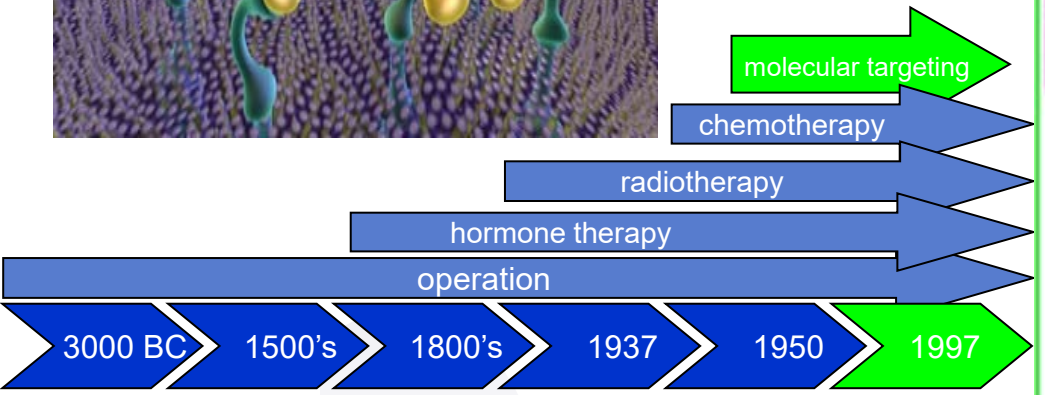
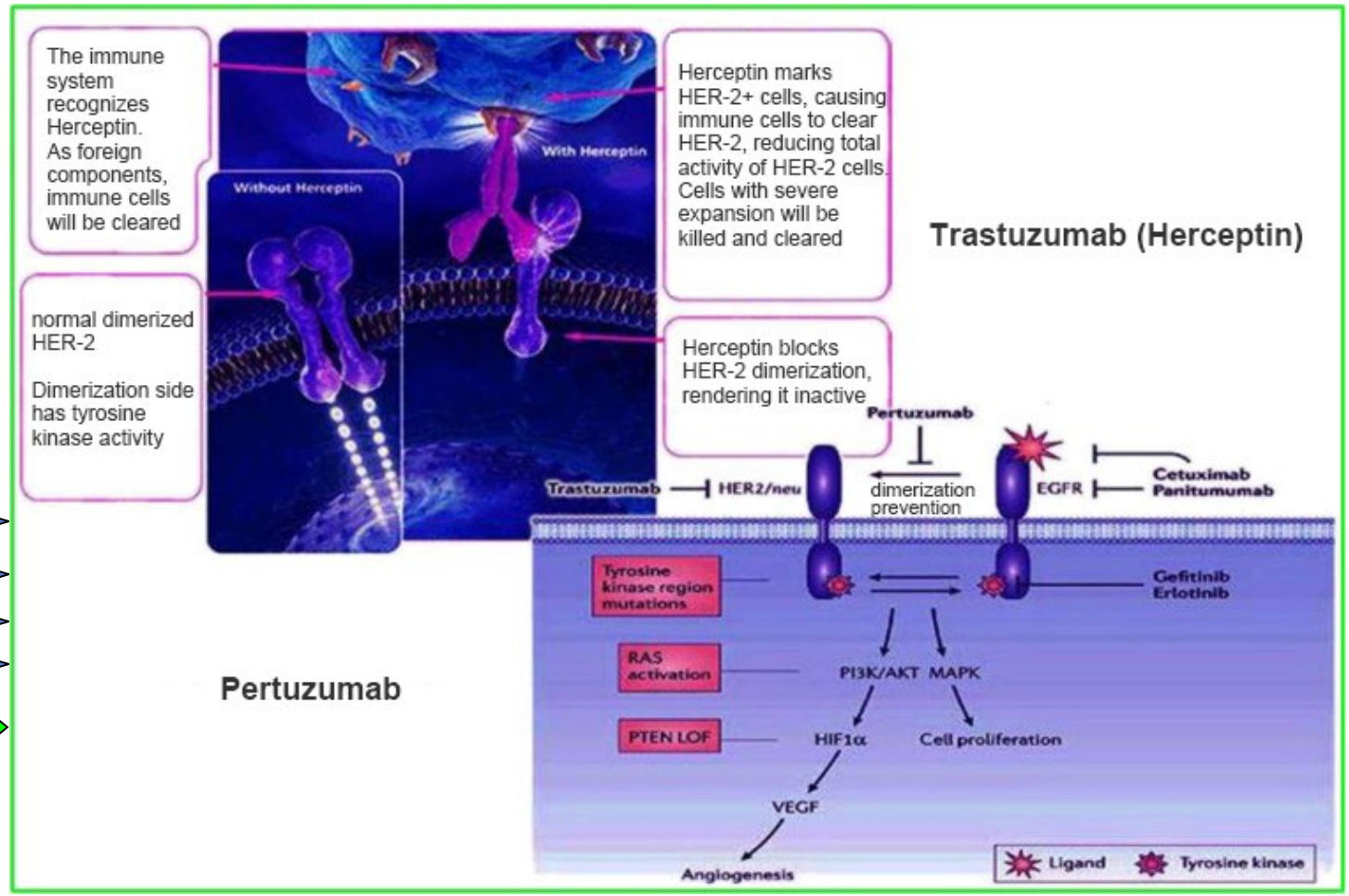
Herceptin brings HER2-positive breast cancer treatment into a new era of molecular targeting







Rayter & Mansi. Medical Therapy of Breast Cancer 2003

Any treatment decision should be based on knowledge of HER2 status

- HER2 overexpression is one of the important factors in the development of certain cancers

  ---HER2 is amplified/overexpressed in both breast and gastric cancer
- HER2 is an independent prognostic factor for breast cancer and gastric cancer
- Anti-HER2 therapy

  ---Prolonged Survival of HER2-Positive Breast Cancer Patients of All Stages

  ---Improving the course of patients with metastatic or recurrent gastric cancer
- Accurate HER2 detection results are of great significance to ensure correct diagnosis and treatment of breast cancer and gastric cancer patients

04 Bio-digital PCR HER2 copy number variation testing system

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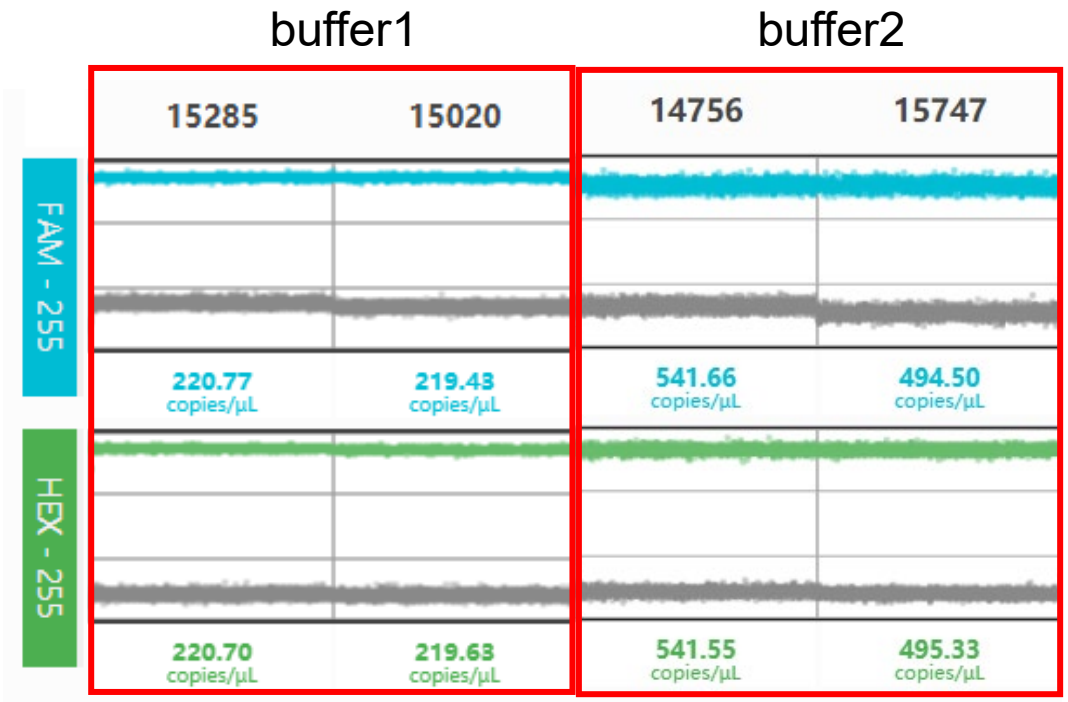
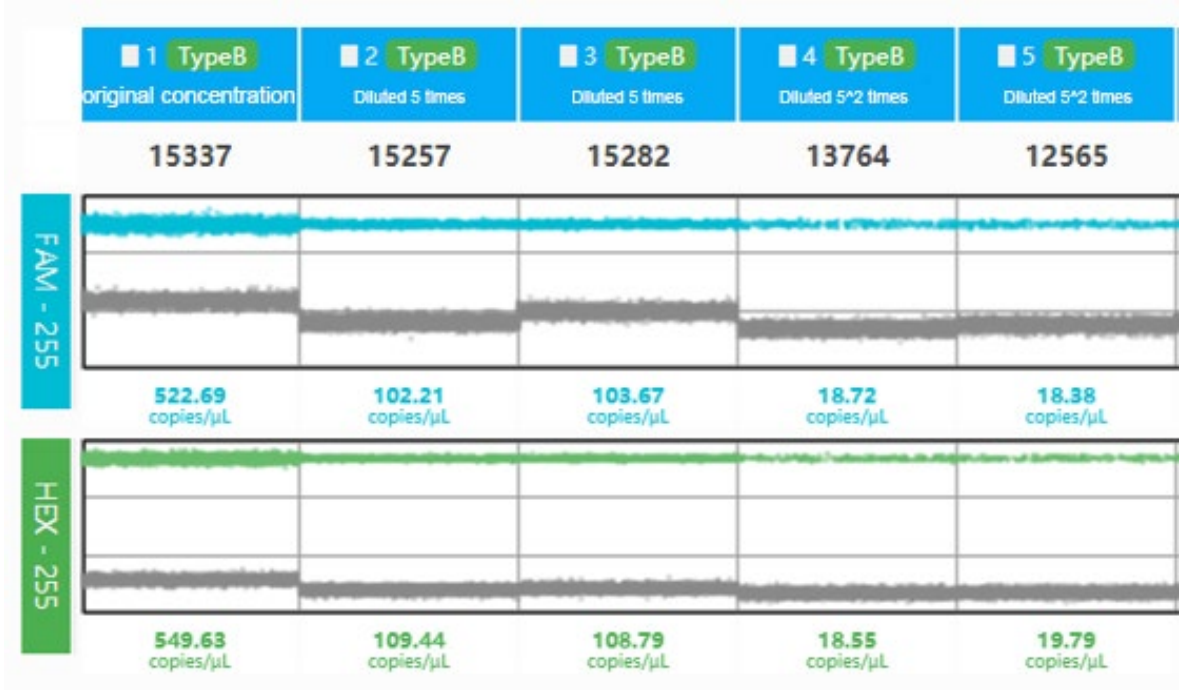
Reference - Genome

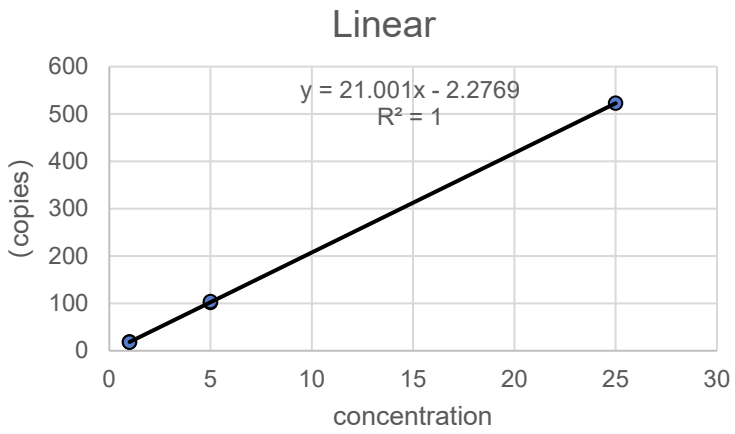


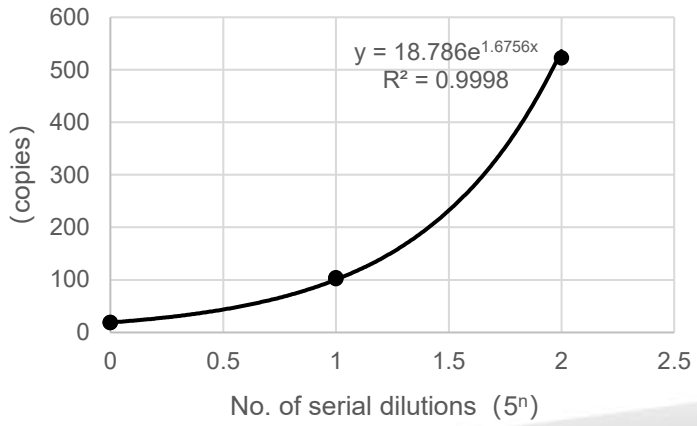
	Buffer1-duplicate1	Buffer1-duplicate2	Buffer2-duplicate1	Buffer2-duplicate2
Copy no. ratio	**220.77/220.70**	**219.43/219.63**	**541.66/541.55**	**494.50/495.33**
FAM/HEX ratio	**1.000**	**0.999**	**1.000**	**0.998**

The experimental results show that the kit can accurately detect negative sample results, and the ratio is relatively stable， and compatible with multiple buffers.

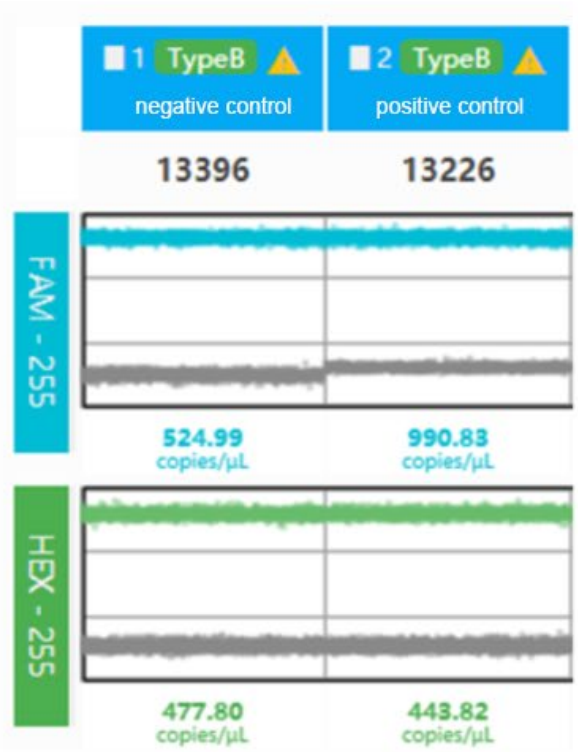
Serial Dilution - Linear

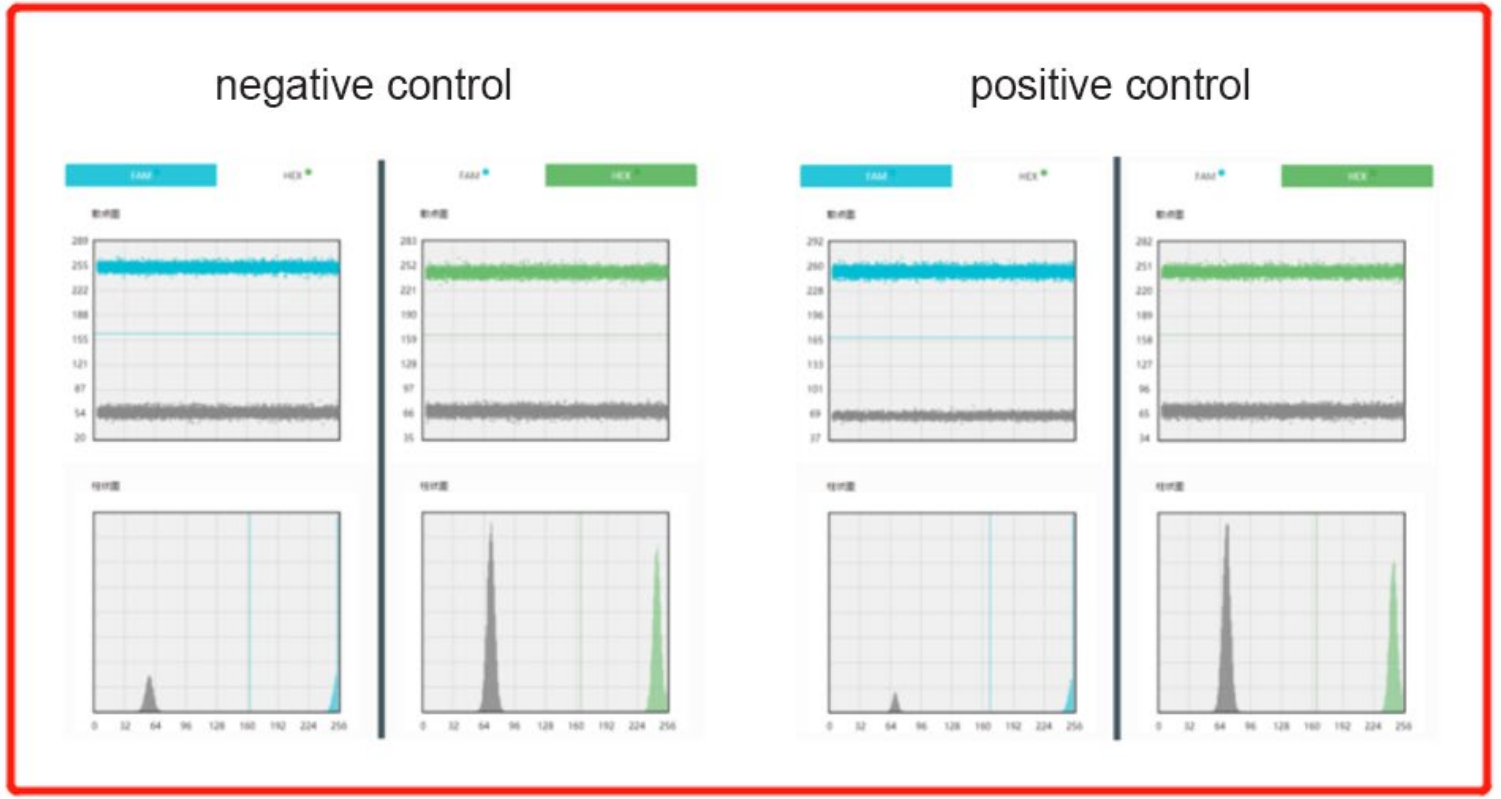






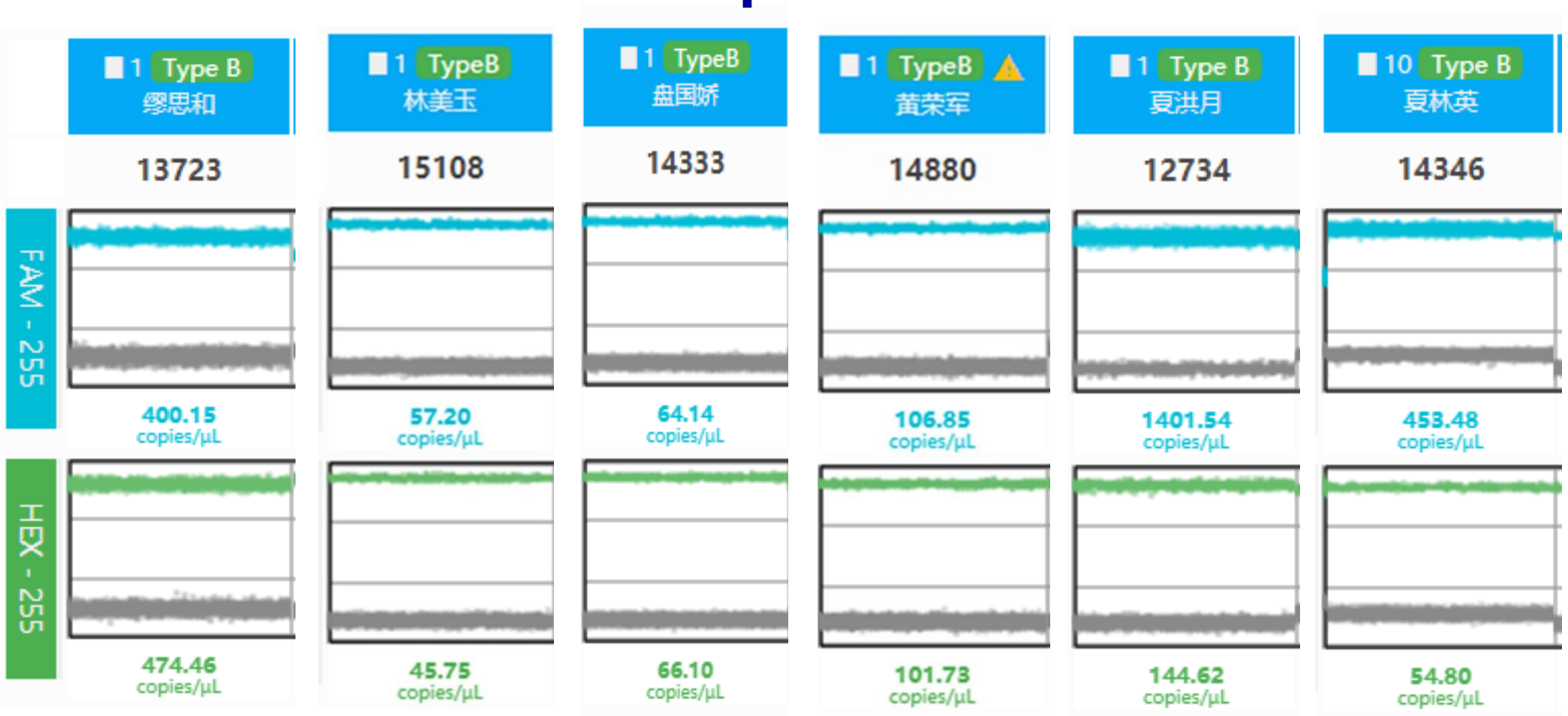
Primer Probe Performance——Meijie kit detection





	copies	Original concentration(copies/uL)	FAM/HEX ratio	result
Negative control *1	524.99/477.80	5249.9/4778	1.09	negative
Positive control *2	990.83/443.82	9908.3/4438.2	2.23	positive

Peripheral Blood Authentic Samples



	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
copies	400.15/474.46	57.20/45.75	64.14/66.10	106.85/101.73	1401.54/144.62	453.48/54.8
FAM/HEX ratio	0.843	1.250	0.970	1.050	9.691	8.275
result	negative	negative	negative	negative	Positive	positive

Question Discussion:

How the immune system works?

Tumor tissue benign?

Treatment for her2 status?
IHC/FISH

THE END

THANKS